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A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Nanshan National Park, Hunan Province, China

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Abstract

A new species of Asian horned toad, *Boulenophrys youran* **sp. nov.**, is described from Nanshan National Park in southwestern Hunan Province, China. Phylogenetic analyses based on mitochondrial 16S rRNA and *COI* genes place it within the *Boulenophrys omeimontis* species group as a distinct lineage. The new species can be diagnosed by the following combination of morphological characters: (1) Body size relatively large, SVL 45.3–51.1 mm in nine adult males, 55.6–58.2 mm in two adult females; (2) head wider than long, males HDL/HDW 0.69–0.83; (3) margin of tongue feebly notched posteriorly; (4) subarticular tubercles present on fingers I, relative finger lengths II < I < IV < III; (5) relative toe lengths: I < V < II < III < IV; (6) vomerine ridges present, vomerine teeth absent; (7) hindlimbs slender, heels overlapping when hindlimbs folded; (8) tibiotarsal articulation exceeding snout tip when leg stretched forward; (9) toes with weak webbing, formula: I1⅓ – 2II1⅓ – 2½III2 – 3½IV3½ – 2V, lateral fringes on toes wide; (10) dense moderate black conical nuptial spines on inner two fingers in breeding males.

Key Words

Boulenophrys youran, diversity, morphology, phylogeny, taxonomy

Introduction

The generic classification within subfamily Megophryinae Bonaparte, 1850 has undergone substantial generic reclassification in recent years (Chen et al. 2017; Mahony et al. 2017; Liu et al. 2018; Dubois et al. 2021; Qi et al. 2021; Lyu et al. 2023), and currently comprises 151 recognized species (Frost 2025). This research adopts the classification of Lyu et al. (2023), which recognizes ten genera. Among these, the genus *Boulenophrys* Fei, Ye & Jiang, 2016 includes 79 species inhabiting tropical and subtropical montane forests across a vast Asian range, from southern China and the Himalayas to Vietnam, Laos, Thailand,

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Myanmar, with less certainty to Bhutan and northeastern India (Fei and Ye 2016; Frost 2025). Three species groups are recognized within *Boulenophrys*: the *B. minor*, *B. omeimontis*, and *B. boettgeri* groups (Qi et al. 2021). Recent studies have revealed extensive diversity within the genus, particularly in southern China, where morphological convergence has frequently confounded species delimitation (Liu et al. 2018). The Hunan Province in Central Southern China exemplifies this diversity, where a total of 19 *Boulenophrys* species has been recorded, and most within the last five years, highlighting the previously underestimated diversity in the region (Shen et al. 2014; Gao et al. 2022; Xiang et al. 2024; Kuang et al. 2025).

During herpetological surveys in Nanshan National Park, southwestern Hunan Province from 2019 to 2025, twelve specimens of *Boulenophrys* were collected from Chengbu Miao Autonomous County and Xinning County. Morphologically, these specimens exhibited traits that assign them to the *B. omeimontis* species group, including: (1) small horn-like tubercles along the dorsolateral margin of the upper eyelid; (2) relatively smooth dorsal skin bearing distinct black spines; and (3) obvious vocal sac morphology (Lyu et al. 2023). Integrative taxonomic evidence based on morphology and phylogeny confirms that these specimens represent a new species, and it is described herein.

Materials and methods

Sampling and morphometrics

Field surveys were conducted in Nanshan National Park (26°01'55"–26°21'32"N, 109°59'25"–110°33'36"E), Hunan Province, China, during April, May, and September from 2023 to 2025. A total of eleven adult and one juvenile *Boulenophrys* specimens were collected (Fig. 1). Muscle tissue samples were excised from each individual upon collection and preserved in 95% ethanol for molecular analyses, while whole specimens were preserved in 75% ethanol. All voucher specimens are deposited in the Animal Museum of the College of Life Sciences, Hunan Normal University (HUNL), Changsha, China. Surveys were permitted by the Administrations of Nanshan National Park, and all procedures were approved by the Animal Ethical and Welfare Committee of Hunan Normal University (No. 2024728).

We measured twelve specimens of the undescribed species, nine adult males, two adult females, one juvenile male, (Suppl. material 1: table S1). Comparative morphological data for *B. dalaolingensis*, *B. sangzhiensis*, *B. fanjingmontis*, *B. qianbeiensis*, *B. spinata* and *B. yezhongensis* were obtained from literature (Table 1) and through examined topotype or type specimens (Appendix 1).

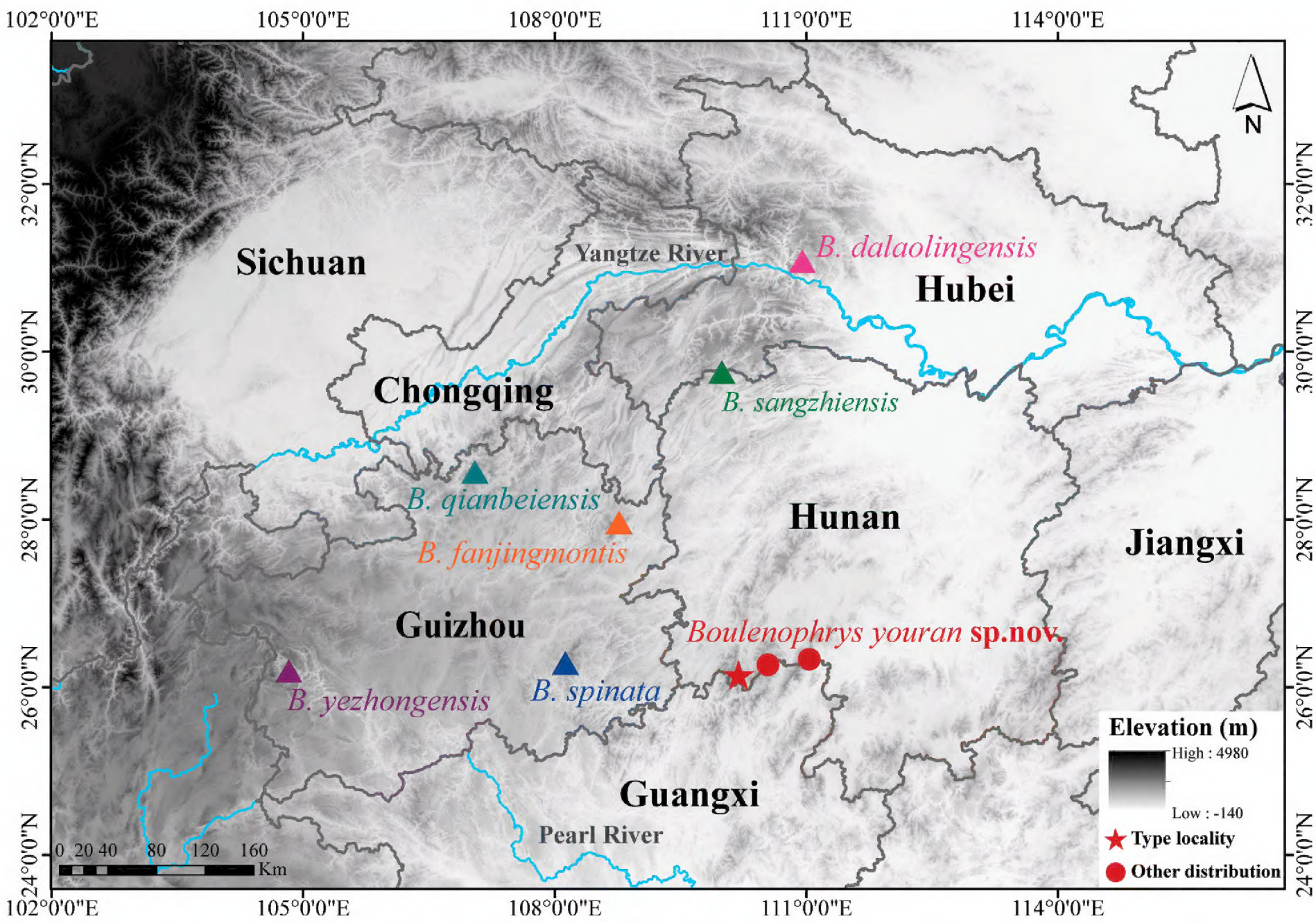

Figure 1. Distribution map for *Boulenophrys youran* sp. nov. and its related species across China. Distribution data accessed from Su et al. (2020), Lyu et al. (2023), Liu et al. (2025a), Wang et al. (2025b). This illustration was created and provided by Bei Xiao.

Table 1. References for morphological characters for congeners of the genus *Boulenophrys*.

ID	Species	References
1	*B. acuta* Wang, Li & Jin, 2014	Li et al. 2014
2	*B. angka* Wu, Suwannapoom, Poyarkov, Pawangkhanant, Xu, Jin, Murphy & Che, 2019	Wu et al. 2019
3	*B. anlongensis* Li, Lu, Liu & Wang, 2020	Li et al. 2020
4	*B. baishanzuensis* Wu, Li, Liu, Wang & Wu, 2020	Wu et al. 2020
5	*B. baolongensis* Ye, Fei & Xie, 2007	Ye et al. 2007
6	*B. binchuanensis* Ye & Fei, 1995	Fei and Ye 2016
7	*B. binlingensis* Jiang, Fei & Ye, 2009	Fei and Ye 2016
8	*B. boettgeri* Boulenger, 1899	Boulenger, 1899
9	*B. brachykolos* Inger & Romer, 1961	Fei and Ye 2016
10	*B. caobangensis* Nguyen, Pham, Nguyen, Luong & Ziegler, 2020	Nguyen et al. 2020
11	*B. caudoprocta* Shen, 1994	Fei and Ye 2016
12	*B. changyangensis* Li, Shi, Liu, Zhao, Gao & Wang, 2025	Li et al. 2025
13	*B. cheni* Wang & Liu, 2014	Wang et al. 2014
14	*B. chishuiensis* Xu, Li, Liu, Wei & Wang, 2020	Xu et al. 2020
15	*B. congjiangensis* Luo, Wang, Wang, Lu, Wang, Deng & Zhou, 2021	Luo et al. 2021
16	*B. daiyunensis* Lyu, Wang & Wang, 2021	Lyu et al. 2021
17	*B. dalaolingensis* Wang, Chen & Liu, 2025	Wang et al. 2025b
18	*B. daoji* Lyu, Zeng, Wang & Wang, 2021	Lyu et al. 2023
19	*B. daweimontis* Rao & Yang, 1997	Fei and Ye 2016
20	*B.daxuemontis* Liu, Li, Cheng, Wei, Wang & Cheng, 2025	Liu et al. 2025b
21	*B. dongguanensis* Wang & Wang, 2019	Wang et al. 2019a
22	*B. dupanglingensis* Xiao & Mo, 2025	Xiao et al. 2025
23	*B. elongata* Zeng, Wang, Chen, Xiao, Zhan, Li & Lin, 2024	Zeng et al. 2024
24	*B. fanjingmontis* Zhang, Liang, Ran & Shen, 2012	Zhang et al.2012
25	*B. fansipanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018a
26	*B. fengshunensis* Wang, Zeng, Lyu, & Wang, 2022	Wang et al. 2022
27	*B. frigida* Tapley, Cutajar, Nguyen, Portway, Mahony, Nguyen, Harding, Luong & Rowley, 2021	Tapley et al. 2020
28	*B. gaolanensis* Song, Wang, Qi, Wang & Wang, 2024	Song et al. 2024
29	*B. gutu* Kang, Wei & Shi, 2025	Kuang et al. 2025
30	*B. hengshanensis* Qian, Hu, Mo, Gao, Zhang & Yang, 2023	Qian et al. 2023
31	*B. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018b
32	*B. huangniushiensis* Wang, Wu, Song, Huang, Zhuo & Wang, 2025	Wang et al. 2025a
33	*B. hungtai* Wang, Zeng, Lyu, Xiao, & Wang, 2022	Wang et al. 2022
34	*B. insularis* Wang, Liu, Lyu, Zeng & Wang, 2017	Wang et al. 2017a
35	*B. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
36	*B. jingdongensis* Fei & Ye, 1983	Fei and Ye 1983
37	*B. jinggangensis* Wang, 2012	Wang et al. 2012
38	*B. jiulianensis* Wang, Zeng, Lyu & Wang, 2019	Lyu et al. 2023
39	*B. kuatunensis Pope, 1929*	Fei and Ye 2016
40	*B. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018
41	*B. lichun* Lin, Chen, Li, Peng, Zeng & Wang, 2024	Lin et al. 2024
42	*B. lushanensis* Shen, Zhou & Li, 2025	Shen et al. 2025a
43	*B. lushuiensis* Shi, Li, Zhu, Jiang, Jiang & Wang, 2021	Shi et al. 2021
44	*B. liboensis* Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017	Zhang et al. 2017
45	*B. lini* Wang & Yang, 2014	Wang et al. 2014
46	*B. lishuiensis* Wang, Liu & Jiang, 2017	Wang et al. 2017b
47	*B. minor* Stejneger, 1926	Fei and Ye 2016
48	*B. mirabilis* Lyu, Wang & Zhao, 2020	Lyu et al. 2020
49	*B. mufumontana* Wang, Lyu & Wang, 2019	Wang et al. 2019a
50	*B. nankunensis* Wang, Zeng & Wang, 2019	Wang et al. 2019a
51	*B. nanlingensis* Lyu, Wang, Liu & Wang, 2019	Wang et al. 2019a
52	*B. nebulosa* Shen & Li, 2025	Shen et al. 2025b
53	*B. obesa* Wang, Li & Zhao, 2014	Li et al. 2014
54	*B. ombrophila* Messenger & Dahn, 2019	Messenger et al. 2019
55	*B. omeimontis* Liu, 1950	Fei and Ye 2016
56	*B. palpebralespinosa* Bourret, 1937	Fei and Ye 2016

ID	Species	References
57	*B. pepe* Wang & Zeng, 2024	Wang et al. 2024
58	*B. puningensis* Wang, Zeng, Lyu, Xiao, & Wang, 2022	Wang et al. 2022
59	*B. qianbeiensis* Su, Shi, Wu, Li, Yao, Wang & Li, 2020	Su et al. 2020
60	*B. rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017	Tapley et al. 2017
61	*B. sangzhiensis* Jiang, Ye & Fei, 2008	Jiang et al. 2008
62	*B. sanmingensis* Lyu & Wang, 2021	Lyu et al. 2023
63	*B. shimentaina* Lyu, Liu & Wang, 2020	Lyu et al. 2023
64	*B. shuichengensis* Tian & Sun, 1995	Tian et al. 2000
65	*B. shunhuangensis* Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019b
66	*B. spinata* Liu & Hu, 1973	Fei and Ye 2016
67	*B. tongboensis* Wang & Lyu, 2021	Lyu et al. 2023
68	*B. tuberogranulata* Shen, Mo & Li, 2010	Mo et al. 2010
69	*B. wugongensis* Wang, Lyu & Wang, 2019	Wang et al. 2019a
70	*B. wuliangshanensis* Ye & Fei, 1995	Fei and Ye 2016
71	*B. wushanensis* Ye & Fei, 1995	Fei and Ye 2016
72	*B. xiangnanensis* Lyu, Zeng & Wang, 2020	Lyu et al. 2023
73	*B. xianjuensis* Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020
74	*B. xuefengmontis* Lyu & Wang, 2023	Lyu et al. 2023
75	*B. yangmingensis* Lyu, Zeng & Wang, 2020	Lyu et al. 2023
76	*B. yaoshanensis* Qi, Mo, Lyu, Wang & Wang, 2021	Qi et al. 2021
77	*B.yezhongensis* Liu, Feng, Shen, Li, Cheng, Wei, Wang & Su, 2025	Liu et al. 2025a
78	*B. yingdeensis* Qi, Lyu, Wang & Wang, 2021	Qi et al. 2021
79	*B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021	Qi et al. 2021

Measurements were made with a digital calipers to the nearest 0.1 mm. Twenty-one morphological characters following the definitions of Fei et al. (2009) and Mahony et al. (2011), including SVL (snout-vent length); HDL (head length); HDW (head width); SL (snout length); NSD (nasal to snout distance); IND (internasal distance); IOD (interorbital distance); ED (eye diameter); NED (nasal to eye distance); TYD (maximal tympanum diameter); UEW (width of upper eyelid); LAL (length of lower arm and hand); LW (lower arm width); HLL (hindlimb length); THL (thigh length); TL (tibia length); TW (maximal tibia width); TFL(length of foot and tarsus); FL (foot length); IAE (distance between posterior corner of eyes); IFE (distance between anterior corner of eyes). Sex was determined by the secondary sexual characteristics, the presence of vocal sacs and nuptial pads in males, absences in females and juvenile. Detailed comparisons were made with all recognized congeners of *Boulenophrys*, comparative morphological data were obtained from the literature (Table 1, Suppl. material 1: table S2) and specimens examined (Appendix 1).

To reduce allometry effects, the ratio of each character to SVL was calculated and log-transformed for subsequent Principal Component Analysis (PCA) (Lleonart et al. 2000). PCA was performed in R version 4.5.1 (Copyright (C) 2025 The R Foundation for Statistical Computing, Vienna, Austria) based on morphometric characters shared by the undescribed species and its related congeners.

Morphometric differences between the new species and its congeners were assessed using one-way analysis of variance (ANOVA) was conducted to test the significance of the difference in the ratio of each morphometric character to the SVL between the adult males of undescribed species and related congeners, with the significance level set at 0.05.

Molecular phylogenetic analyses

Genomic DNA was extracted from muscle tissue using the DNA extraction kit (Tiangen Biotech Co., Ltd, Beijing). Two mitochondrial gene fragments, the partial sequence of 16S ribosomal RNA gene (16S rRNA) and the cytochrome c oxidase subunit I gene (*COI*), were amplified for phylogenetic reconstruction using primers from Simon et al. (1994) and Meyer et al. (2005), respectively, PCR followed the protocols described in Chen et al. (2017). All amplification products were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

For phylogenetic analysis, we obtained sequences from 77 recognized *Boulenophrys* species, except for *B. lushanensis*, *B. nebulosa* due to data unavailable. And two outgroup taxa (*Xenophrys mangshanensis* and *X. glandulosa*) from GenBank, these were chosen based on their previous use in *Boulenophrys* phylogenies (Suppl. material 1: table S3). Sequences were aligned using MUSCLE (Edgar 2004) with default parameters. Phylogenetic analyses were conducted in PhyloSuite v1.2.3 based on the concatenated sequences of 16S rRNA and *COI* fragments (Zhang et al. 2020). The best-fit partition model GTR+I+G was selected by MODELFINDER v2.2.0 (Kalyaanamoorthy et al. 2017) under the corrected Akaike Information Criterion (AICc). Bayesian inference (BI) was implemented in MrBayes 3.2 (Ronquist et al. 2012) with 5 million generations, sampling every 1×10^3 generations, the first 25% of samples were discarded as burn-in, convergence was assessed by potential scale reduction factor (PSRF) of < 0.01, effective sample sizes (ESS) > 200 for all parameters. Maximum likelihood (ML) analysis was performed in IQ-TREE v2.2.0 (Nguyen et al. 2015) under the TVM+ F+I+I+R4 model, with

5×10^3 ultrafast bootstraps replicates (Minh et al. 2013). Uncorrected *p*-distances among all samples were calculated in MEGA v11 (Tamura et al. 2021).

Skeleton scanning

Skeletal preparations of two adult male specimens of the undescribed species (holotype: HUNL XB25061601; paratype: HUNL XB25061602) were subjected to Micro-CT scanning on U-OIBLT/FLT/CTXUHR (MILabs, Netherlands). Specimens were scanned along the coronal axis at a resolution of 1024 × 1024 pixels in Accurate scan mode, with the X-ray tube set to 55 kV and 0.17 mA. Image reconstruction was performed using MILabs Rebuild software, and three-dimensional morphological analysis was conducted in Imalytics Preclinic 3.0 (Gremse-IT GmbH, Aachen, Germany). Skeleton terminology follows Fei and Ye (2016).

Results

Phylogenetic analyses

Both BI and ML analyses yielded identical tree topologies, with the ML phylogenetic tree shown in Fig. 2. The undescribed species formed a monophyletic clade (BPP = 1.00, BS = 100), clearly divergent from its sister lineage, although some nodes received weaker support. Uncorrected interspecific *p*-distances between the undescribed species and its closest congeners ranged from 3.8% to 5.5% for *COI* (vs. *B. dalaolingensis*: 5.5%; *B. fanjingmontis*: 3.8%; *B. qianbeiensis*: 4.3%; *B. sangzhiensis*: 4.0%; *B. spinata*: 4.2%, *B.yezhongensis*: 5.1%) and from 0.9% to 1.4% for 16S rRNA (vs. *B. dalaolingensis*: 0.9%; *B. fanjingmontis*: 1.1%; *B. qianbeiensis*: 1.4%; *B. spinata*: 1.1%; *B. sangzhiensis*: 1.4%, *B.yezhongensis*: 1.1%) (Suppl. material 1: tables S4, S5).

Skeleton characteristics

All skeleton characters were found to be consistent across the examined specimens, They shared skeletal morphology includes: maxilla overlapping the quadratojugal, with well-developed maxillary teeth and distinct vomerine ridge; nasal bones relatively small, separated from each other, and articulating with the sphenethmoid and frontoparietal; the latter exhibiting an almost uniformly wide anterior and posterior portion with a visible fontanelle; vertically inclined nasal process of the premaxilla; prootic separated from the exoccipital, with the posterior edge aligning with the jaw articulation line; moderately developed pterygoid; eight presacral vertebrae with imbricate neural arches and barely evident neural spines; sacrum with wide, large diapophyses and a monocondylar sacrococcygeal articulation; limb elements including a humerus with developed ridges, radio-ulna bearing a ridge on the inner side, and bluntly pointed terminal phalanges; and a femur slightly longer than the tibia (Fig. 3).

Morphological analyses

PCA Plot of adult male specimens indicated that the first two principal components collectively explained 78.1% of the total morphological variance (Fig. 4). PC1 components explained 68.8% of the variance and were primarily defined by high loadings on HDW, HLL, THL, TFL, FL, IFE and TL, whereas PC2 components explained an additional 9.3% and were most associated with NED, IOD, LW and TW. In the morphospace, the undescribed species was clearly separated from *B. fanjingmontis*, *B. sangzhiensis* and *B. yezhongensis*. It was distinguished from *B. fanjingmontis* and *B. sangzhiensis* primarily along the PC1 axis, and from *B. yezhongensis* along the PC2 axis. Two species, *B. dalaolingensis* and *B. qianbeiensis* could not be distinguished from the undescribed species in the PCA analysis. However, ANOVA revealed that the adult males of undescribed species are significantly different from *B. dalaolingensis* in SVL, HL/SVL, HW/SVL, SL/SVL, IND/SVL and TL/SVL, and *B. qianbeiensis* in SVL, HL/SVL, HW/SVL, ED/SVL, UEW/SVL, LAL/SVL, TW/SVL, FL/SVL, TYD/SVL and IAE/SVL (Table 2). The undescribed species also differs from other related species in some measurements listed in Suppl. material 1: table S2. Additionly, quantitative morphological comparisons further support the distinctiveness of the undescribed species, as detailed in the following section.

Taxonomic account

***Boulenophrys youran* Xiao, Shi & Mo, sp. nov.**

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Figs 5, 6, 7, 8

Type materials. ***Holotype.*** • HUNL XB25061601, adult male, collected by Bei Xiao, Leqiang Zhu, Hongyu Wang on 16 June 2025 from Nanshan National Park (26°9'23.59"N, 110°10'47.32"E, ca 1 040 m a.s.l.), Chengbu Miao Autonomous County, Shaoyang City, Hunan Province, China, (Fig. 5).

Paratypes. (n = 11) • HUNL XB25061602 and HUNL XB25061603, two adult males, collected from the same place as holotype. • HUNL XB24052601-HUNL XB24052603, three males, collected on 26 May 2024 by Bei Xiao, Leqiang Zhu, and Ayinuer Maimaiti; • HUNL LH23051201, adult male, and HUNL LH23051202, adult female, collected from Yaorenping Village (26°14'50.43"N, 110°30'34.74"E, 1 090 m a.s.l.) on 12 May 2023 by Hui Li and Leqiang Zhu; • HUNL DB24042601 and HUNL DB24042602, two adult males, HUNL DB24042603, adult female, HUNL DB23092403, juvenile male, collected from Fanshuijiang Village,

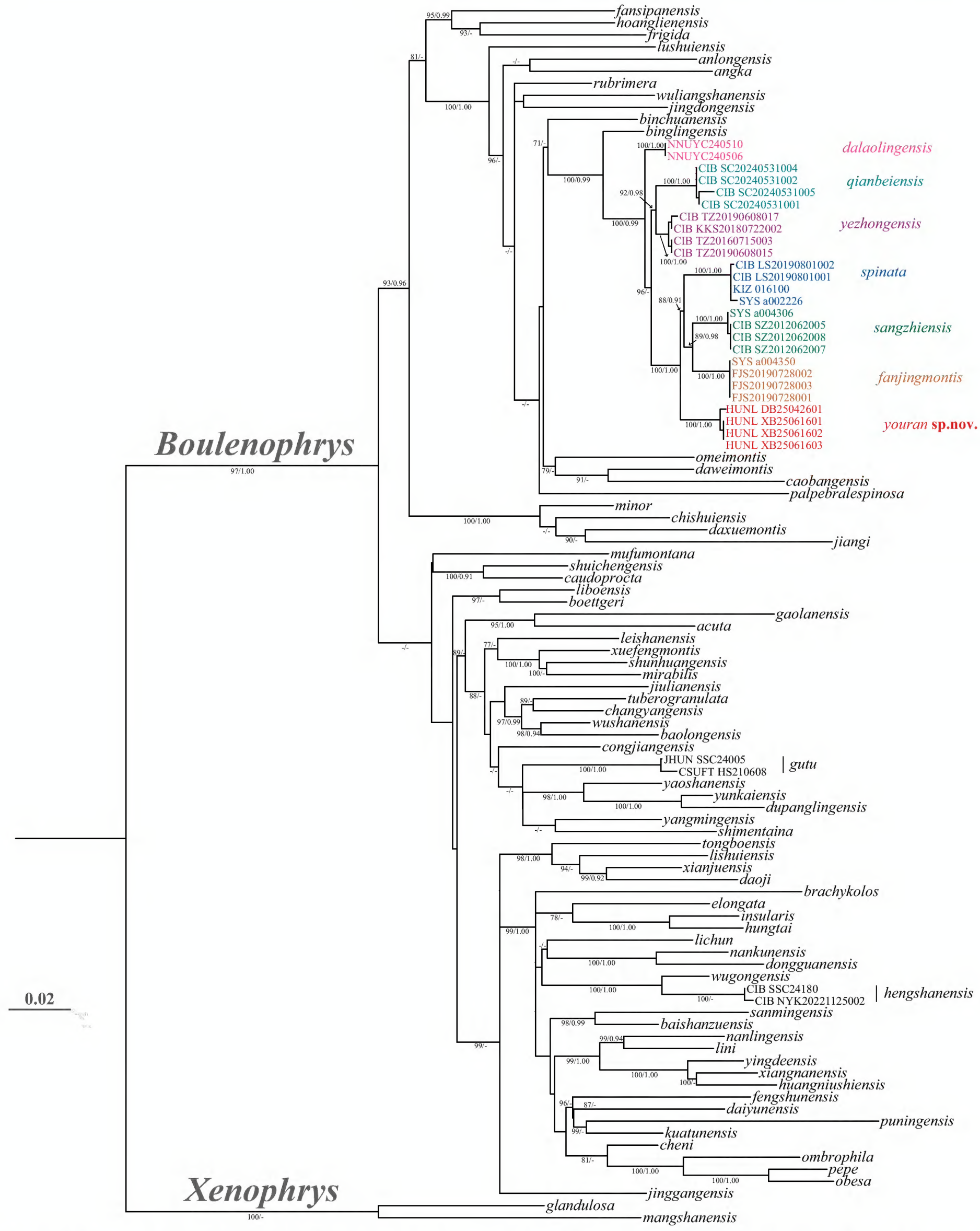

Figure 2. ML tree of the genus *Boulenophrys* reconstructed inferred from 16S rRNA and *COI* gene. Bayesian posterior probability (BPP)/ML bootstrap supports (BS)were denoted beside each node is, a dash (–) denotes BS values below 70% or BPP values below 0.90.

Xinning County, Hunan province (26°23'4.17"N, 111°1'16.86"E, 1 206 m a.s.l.) on 26 April 2024 and 24 September 2023 by Dongbo Fei, Changle Liao.

Etymology. The specific epithet *youran* is a noun in apposition derived from the Chinese word "悠然" (yōu rán), meaning tranquil or composed. It references the verse "采菊东篱下,悠然见南山" from the classical poem Drinking Wine No. 5 by Tao Yuanming (365–427 CE), which depicts a state of serene harmony with nature. The name thus alludes to the species type locality, Nanshan (南山),

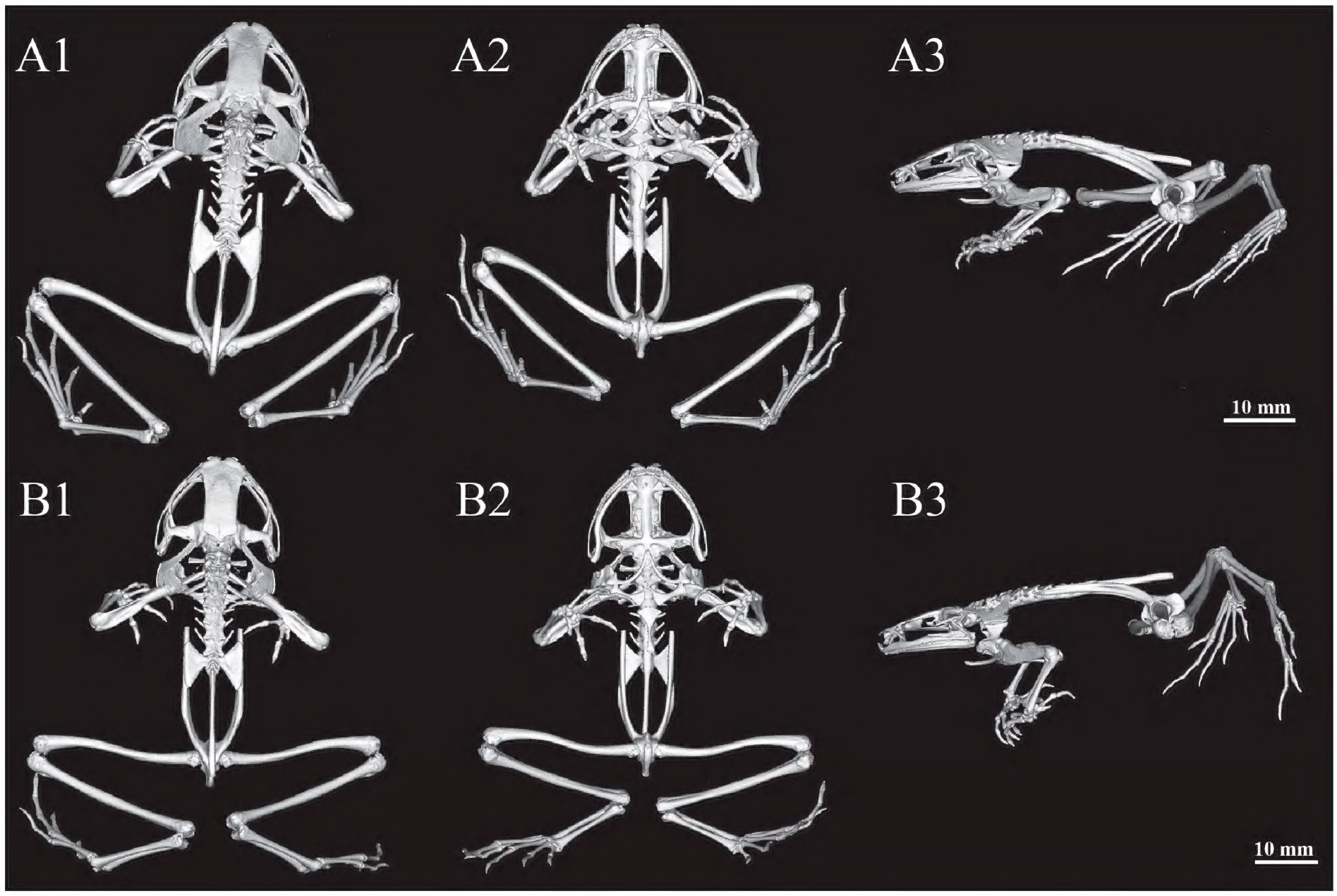

Figure 3. Skeleton of *Boulenophrys youran* sp. nov. in dorsal, ventral and left lateral views. **A1–A3.** Holotype HUNL XB25061601; **B1–B3.** Paratype HUNL XB25061602.

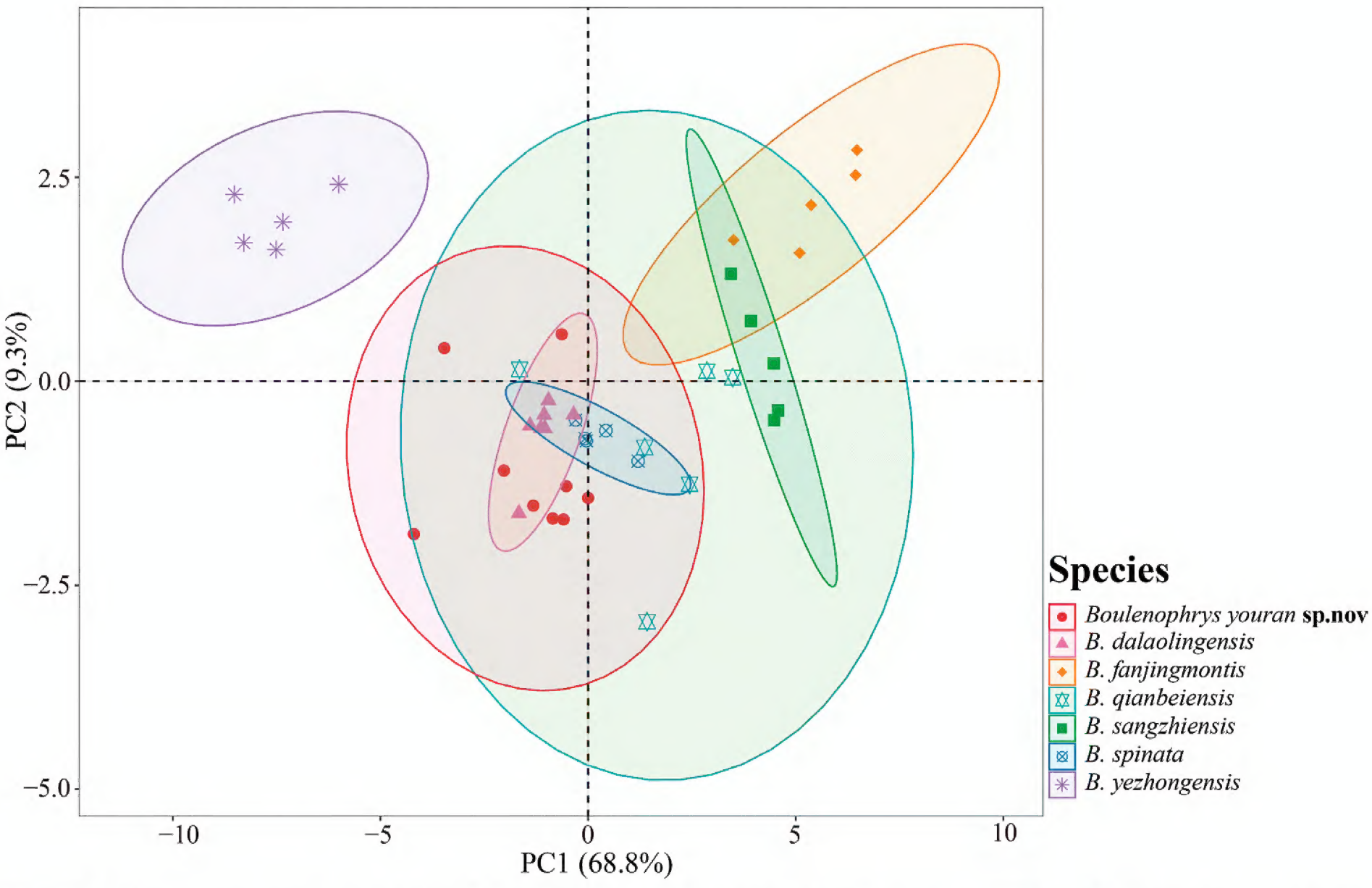

Figure 4. Principal component analysis based on morphometric variation among adult male specimens of *Boulenophrys youran* sp. nov. and closely related congeners.

Table 2. Morphometric comparisons in adult males of *Boulenophrys youran* sp. nov. and related species. *p*-values < 0.05 were present in bold.

Measurements	vs *B. dalaolingensis*	vs *B. fanjingmontis*	vs *B. qianbeiensis*	vs *B. sangzhiensis*	vs *B. spinata*	vs *B. yezhongensis*
SVL	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	0.62
HL/SVL	**0.01**	0.18	**0.01**	0.33	0.91	**0.00**
HW/SVL	**0.00**	**0.01**	**0.00**	0.07	0.19	0.52
SL/SVL	**0.00**	0.07	0.13	0.42	0.14	0.15
NED/SVL	NA	0.17	0.57	0.96	0.47	**0.01**
NSD/SVL	NA	**0.01**	0.12	0.99	0.85	**0.00**
IND/SVL	**0.00**	**0.01**	0.20	0.41	0.22	**0.00**
IOD/SVL	0.22	0.40	0.68	0.21	0.51	0.32
ED/SVL	0.16	0.90	**0.00**	0.10	0.69	0.62
UEW/SVL	NA	0.48	**0.00**	0.06	0.71	0.08
LAL/SVL	0.55	0.07	**0.00**	**0.04**	0.07	**0.00**
LW/SVL	NA	**0.01**	0.07	0.32	0.25	**0.00**
HLL/SVL	NA	0.11	0.23	0.71	0.63	**0.00**
THL/SVL	NA	0.25	0.65	0.19	0.33	**0.00**
TL/SVL	**0.00**	**0.02**	0.22	**0.05**	0.37	**0.00**
TW/SVL	NA	**0.01**	**0.01**	0.47	0.63	**0.00**
TFL/SVL	NA	**0.02**	0.16	0.20	0.67	**0.00**
FL/SVL	0.00	0.39	**0.01**	0.33	**0.02**	**0.00**
TYD/SVL	**0.01**	0.31	**0.00**	**0.04**	0.78	**0.00**
IFE/SVL	NA	**0.02**	0.37	**0.02**	**0.03**	0.06
IAE/SVL	NA	**0.02**	**0.03**	0.34	**0.03**	0.62

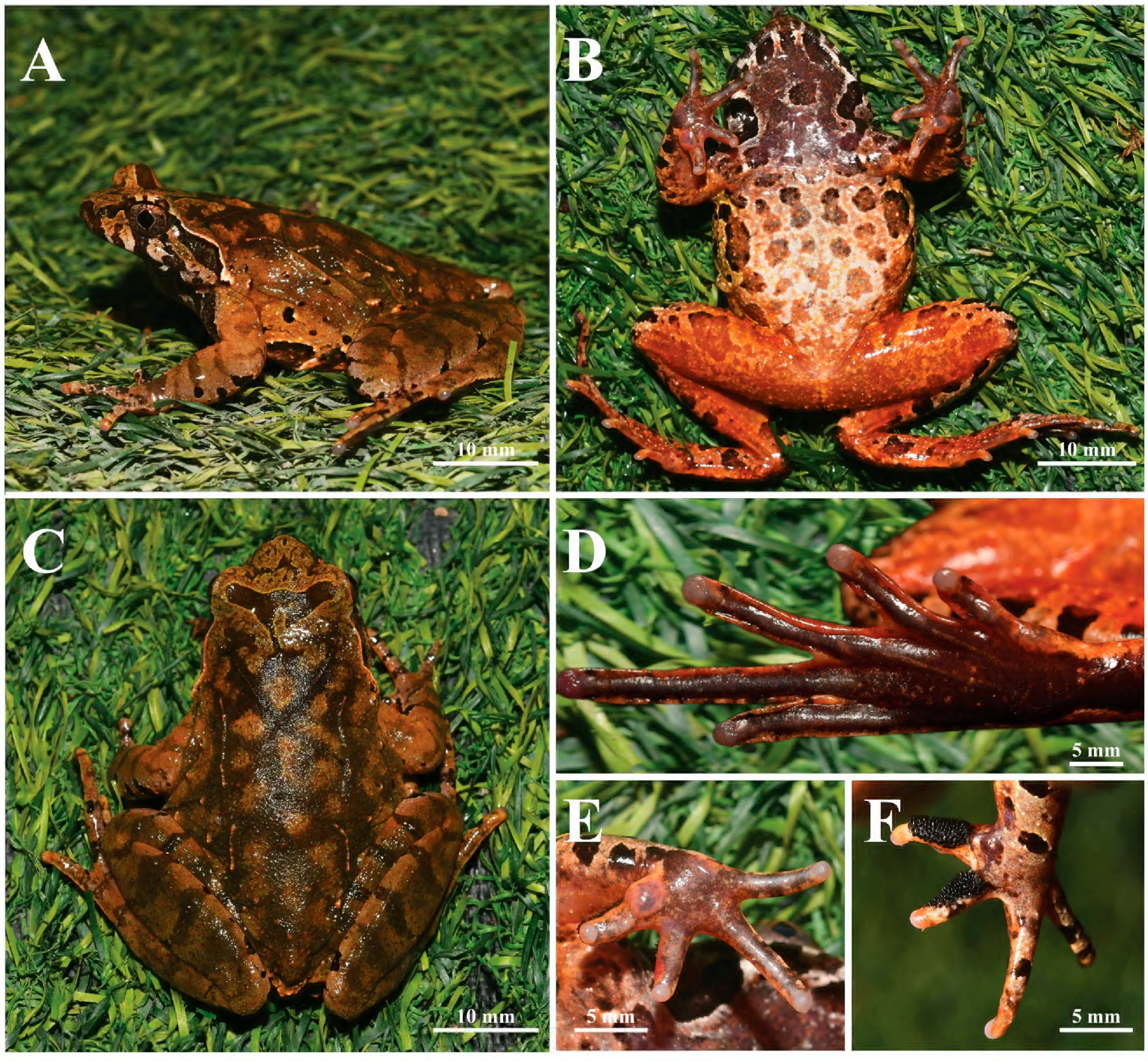

Figure 5. Holotype of *Boulenophrys youran* sp. nov. in life (HUNL XB25061601). **A.** Lateral view; **B.** Ventral view; **C.** Dorsal view; **D.** Plantar view of right foot; **E.** Volar view of right hand; **F.** Dorsal view of left hand. Photos by Leqiang Zhu.

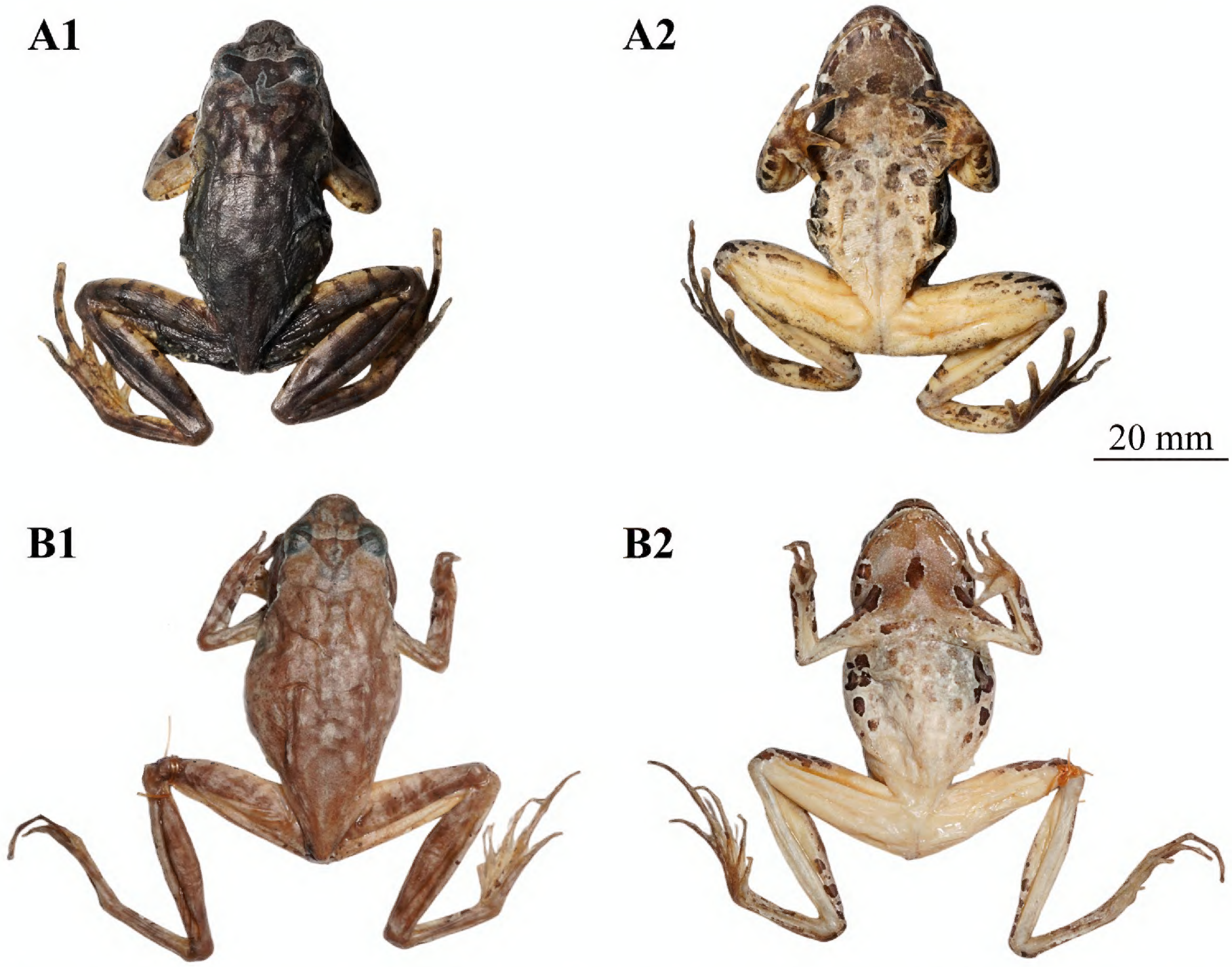

Figure 6. *Boulenophrys youran* sp. nov. in preservation. **A.** Adult male, holotype HUNL XB25061601; **B.** Adult female, paratype HUNL LH23051202; 1. dorsal view; 2. ventral view. Photos by Shengchao Shi.

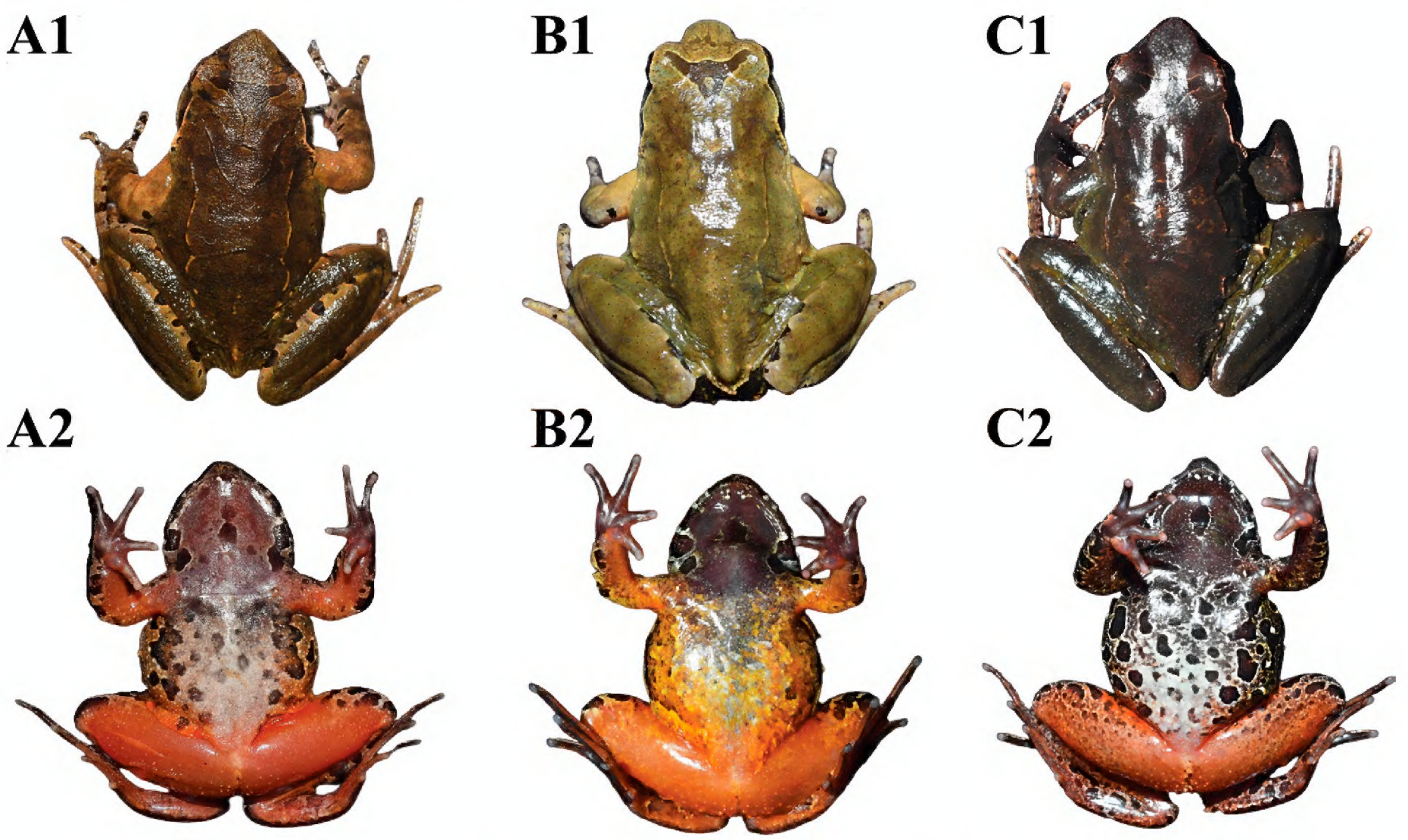

Figure 7. Coloration variations of *Boulenophrys youran* sp. nov. in adult males. Specimens: **A.** HUNL XB25061603; **B.** HUNL XB24052601; **C.** HUNL XB24052602; 1. dorsal view; 2. ventral view. Photos by Hui Li.

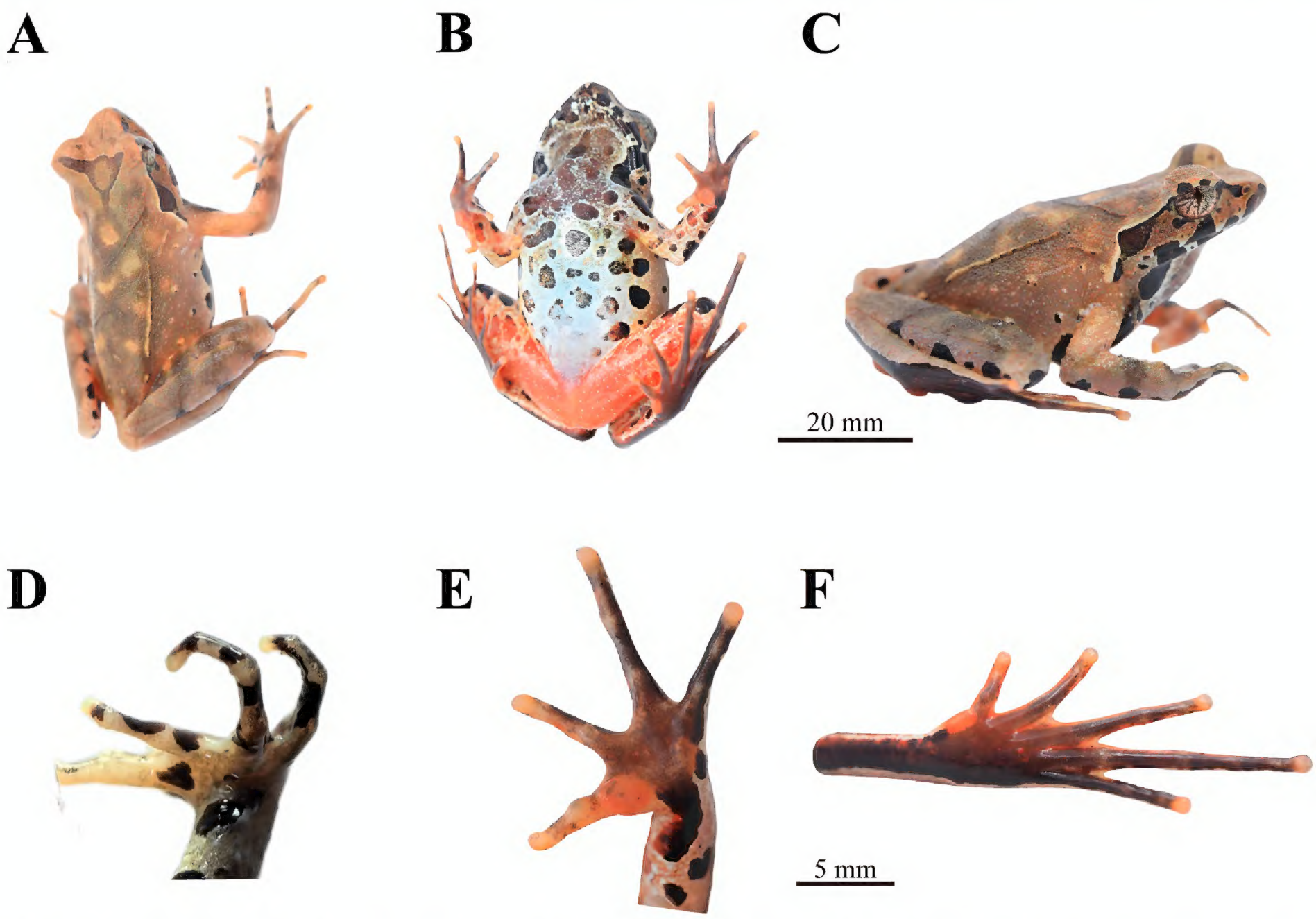

Figure 8. Juvenile of *Boulenophrys youran* sp. nov. (HUNL DB23092403) in life and preservation. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view; **D.** Dorsal view of hand (in preservation); **E.** Volar view of hand; **F.** Plantar view of foot. Photos by Dongbo Fei.

and evokes the pristine, undisturbed montane streams it inhabits. The English vernacular name "Youran Horned Toad" is here proposed, with the corresponding Chinese name "悠然角蟾" (Yōu Rán Jiǎo Chán).

Diagnosis. This species can be distinguished from its congeners by the following combination of morphological characters: (1) Body size relatively large, SVL 45.3–51.1 mm in nine adult males, 55.6–58.2 mm in two adult females; (2) head wider than long, males HDL/HDW 0.69–0.83; (3) margin of tongue feebly notched posteriorly; (4) subarticular tubercles present on fingers I, relative finger lengths II < I < IV < III; (5) relative toe lengths: I < V < II < III < IV; (6) vomerine ridges present, vomerine teeth absent; (7) hindlimbs slender, heels overlapping when hindlimbs folded; (8) tibiotarsal articulation exceeding snout tip when leg stretched forward; (9) toes with weak webbing, formula: I1⅓ – 2II1⅓ – 2½III2 – 3½IV3½ – 2V, lateral fringes on toes wide; (10) dense moderate black conical nuptial spines on inner two fingers in breeding adult males.

Description of holotype. HUNL XB25061601, adult male with relatively large body size, SVL 51.1 mm; head wider than long, HL/HW 0.80; snout pointed in dorsal view, projecting and obliquely sloping in profile, distinctly protruding beyond the lower jaw; top of head flat; eyes moderate in size, ED/HDL 0.34, pupil vertically diamond-shaped; nostril lateral, closer to snout than to eye, loreal region concave, canthus rostralis distinct; internasal distance slightly larger than interorbital distance; tympanum moderate in size with an distinct margin, TD/ED 0.62; supratympanic fold prominent, gradually enlarged posteriorly; choanae large, ovoid, located at base of the maxilla; vomerine ridge present and vomerine teeth absent, maxillary teeth present; tongue notched posteriorly; a large single internal subgular vocal sac present with slit like openings on mandible at corner of mouth.

Forelimbs short and robust; forearm length shorter than hand length, lower arm and hand length 0.41 of SVL; relative finger lengths: III > IV > II > I; fingertips slightly dilated and rounded, without lateral fringes, subarticular tubercles only on fingers I, absent on fingers II – IV, replaced by low callous dermal ridges; inner metacarpal tubercle prominently enlarged, outer metacarpal tubercle smaller and flattened. Dense moderate black conical nuptial spines present on inner two fingers. Hindlimbs slender, length approximately 1.7 times of SVL, tibia length 0.56 of SVL, thigh length shorter than tibia length, but greater than foot length; heels overlapping when flexed hindlimbs are held at right angles to the body axis; tibiotarsal articulation exceeding snout tip when leg stretched forward; relative toes length: I < V < II < III < IV; toe tips rounded and slightly expanded; subarticular tubercles indistinct, toes bear approximately one-third webbing and possess wide lateral fringes; inner metatarsal tubercle ovoid, outer one absent.

Dorsal skin rough, bearing numerous granules tipped with black spines; several large warts scattered on flanks; small horn-like tubercle at the edge of each upper eyelid;

dense, tiny black spines present on the skin of upper lip, upper eyelid, tympanum, loreal and temporal regions; weakly V-shaped skin ridge and two discontinuous dorsolateral parallel ridges along the mid-dorsum; dorsal surfaces of hindlimbs with small black spines, and distinct transverse bands; ventral skin smooth, large pectoral glands located close to the axilla; numerous white granules on the shanks and thighs; femoral glands small and slightly raised, positioned near cloaca on the posterior surface of each thigh.

Coloration of holotype. In life, dorsum yellowish brown with irregular dark-brown patches; a butterfly-shaped marking with a light edge between the eyes; a dark vertical stripe below the eyes, iris copper-brown; temporal region divided into two-toned triangular patches; lips with alternating black and white vertical bars; throat and anterior chest purple-brown, with three black longitudinal stripes edged in white centrally and laterally; posterior flanks orange with white spots and black blotches; groin, inner thighs, and shank surfaces red-orange, tarsus and metatarsus black; palms and soles uniformly brown, subarticular and metacarpal tubercles pinkish; forearms with scattered black patches dorsally; limb transverse bands distinct, with four fine transverse bands visible on the thigh and tibia.

In preservative (Fig. 6), the dorsum faded to grayish brown with more clearly defined black spots and patches; butterfly-shaped marking on the head solid dark brown; forearm bears two oblique black bands, hindlimbs grayish with four dark transverse bands; pectoral and femoral glands appear grayish white; ventral orange mottling is absent, feet ventrally gray-brown with dark blotches near vent; posterior ventral, inner thighs, and upper tibia milky yellow; tips of fingers and toes, subarticular tubercles, both inner and outer metacarpal tubercles all milky yellow.

Variations. Females (SVL 55.6–58.2 mm, $n = 2$) are larger than males (SVL 45.5–51.1 mm, $n = 9$). Paratypes match the holotype in diagnostic morphological characters but exhibit considerable variation in dorsal and ventral coloration (Fig. 7). However, male HUNL XB24052601 displays a dark-brown to black dorsum and a grayish-white venter with irregular dark patches on the chest; while HUNL XB24052602 has dorsal surface olive green without dark-brown patches. Dense moderate black conical nuptial spines on inner two fingers and a single internal subgular vocal sac present in breeding males. The lateral fringes of toes are well-developed in males but less distinct in females and the juvenile specimen HUNL DB23092403 (Fig. 8). Detailed measurements are provided in Suppl. material 1: table S1.

Comparisons. *Boulenophrys youran* sp. nov. is assigned to the *B. omeimontis* group based on phylogenetic results (Fig. 2), and is phylogenetically closest to, *B. dalaolingensis*, *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, *B. spinata* and *B. yezhongensis*. Detailed comparisons with its phylogenetically close congeners are provided below (Figs 9, 10, Table 2).

The new species is easily distinguished from *Boulenophrys dalaolingensis* by the following characters: (1) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the posterior corner of the eye in the latter); (2) head wider than long, males HDL/HDW 0.69–0.83 (vs. 0.85–0.98 in the latter) (Fig. 9B); (3) dense moderate nuptial spine on inner two fingers of breeding males (vs. larger nuptial spine in the latter) (Fig. 10A5, B5). The results of One-way ANOVA showed that *Boulenophrys youran* sp. nov. was significantly different from *B. fanjingmontis* in HL/SVL, HW/SVL, SL/SVL, IND/SVL, TL/SVL and TYD/SVL.

The new species is easily distinguished from *Boulenophrys fanjingmontis* by the following characters: (1) smaller body size: in adult males, the SVL is 45.3–51.1 mm (vs. 58.2–63.6 mm), and in adult females, the SVL is 55.6–58.6 mm (vs. 62.8–72.2 mm); (2) dense moderate nuptial spines on inner two fingers in breeding males (vs. smaller and denser nuptial spines) (Fig. 10A5, B5); (3) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the anterior corner of the eye in the latter). The results of One-way ANOVA showed that *Boulenophrys youran* sp. nov. was significantly different from *B. fanjingmontis* in HW/SVL, NSD/SVL, TL/SVL, IFE/SVL and IAE/SVL.

The new species is easily distinguished from *Boulenophrys qianbeiensis* by the following characters: (1) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the posterior corner of the eye); (2) vomerine teeth absent (vs. vomerine teeth present); (3) horn-like tubercle present at the edge of each upper eyelid (vs. absent); (4) dense moderate nuptial spines on inner two fingers of breeding males (vs. smaller and denser). The results of One-way ANOVA showed that *B. youran* sp. nov. was significantly different from *B. qianbeiensis* in HL/SVL, HW/SVL, ED/SVL, UEW/SVL, TW/SVL, FL/SVL, IAE/SVL and TYD/SVL.

The new species differs from *Boulenophrys spinata* by the following characters: (1) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the anterior corner of the eye); (2) webbing between toes weak (vs. webbing prominent) (Fig. 10A3, E3). The results of One-way ANOVA showed that *B. youran* sp. nov. was significantly different from *B. spinata* on HW/SVL, NED/SVL, IND/SVL, IAE/SVL, IFE/SVL.

The new species differs from *Boulenophrys sangzhiensis* by the following characters: (1) having a slightly smaller body size, in adult males SVL 45.3–51.1 mm (vs. 55.7–62.2 mm in the latter), in adult females SVL 55.6–58.6 mm (vs. 62.2–76.3 mm in the latter); (2) dense moderate nuptial spines on inner two fingers of breeding males (vs. small and dense) (Fig. 10A5, F5); (3) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the anterior corner of the eye). The results of One-way ANOVA showed that *B. youran* sp. nov. was significantly different from *B. sangzhiensis* on HW/SVL, UEW/SVL, LAL/SVL, TYD/SVL and IFE/SVL.

The new species differs from *Boulenophrys yezhongensis* by the following characters: (1) dense moderate nuptial spines on inner two fingers of breeding males (vs. smaller and dense distributed in the latter) (Fig. 10A5, G5); (2) toes with wide lateral fringes and weak webbing (vs. lateral fringes absent and rudimentary webbing); (3) tongue feebly notched posteriorly (vs. tongue not notched posteriorly); (4) tibiotarsal articulation exceeding snout tip when leg stretched forward (vs. to the middle of the eye in the latter).

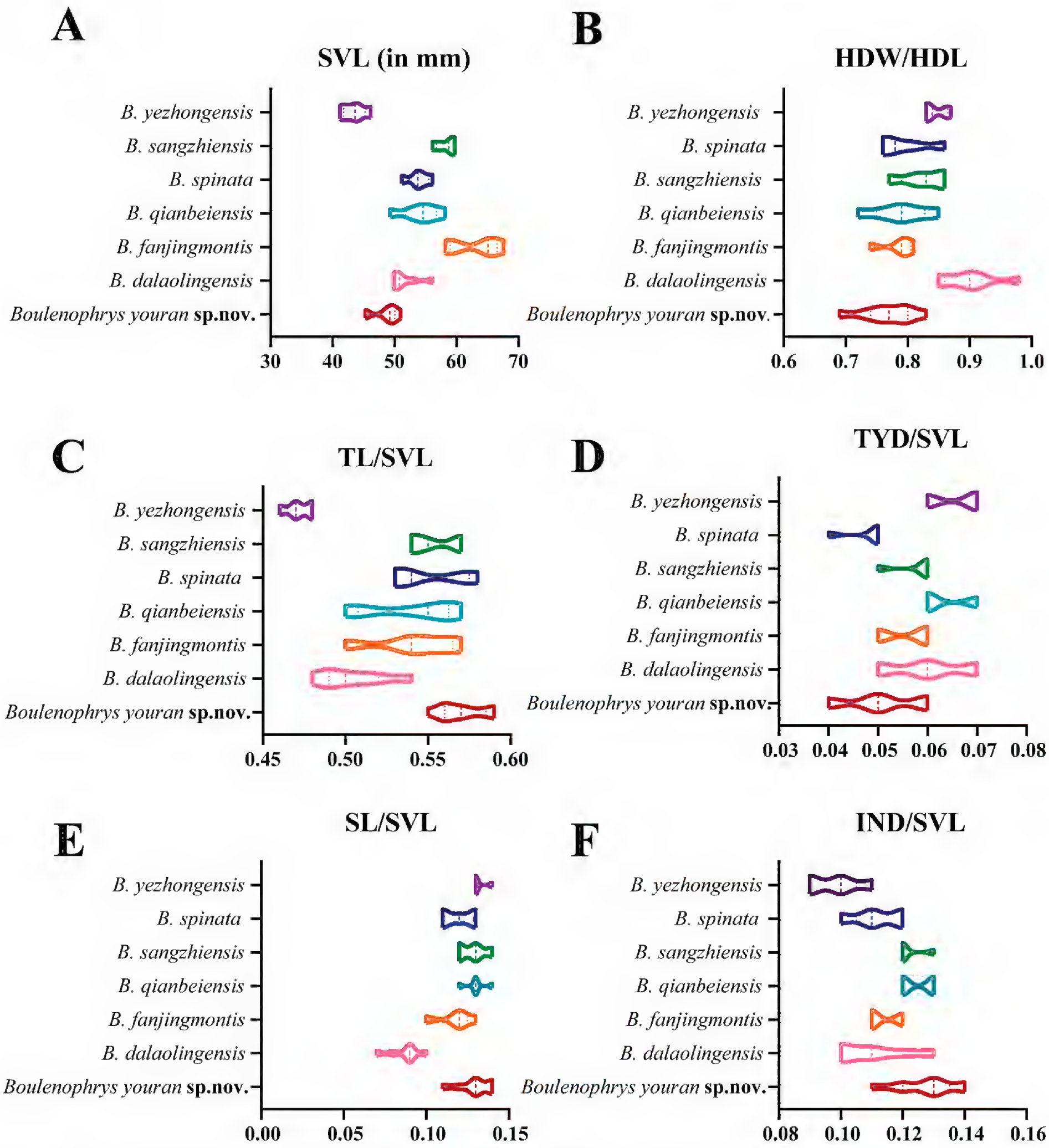

Figure 9. Violin-plot showing the difference between *Boulenophrys youran* sp. nov. and its close congeners.

The new species has a moderate body size, with SVL 45.3–51.1 mm in males, 55.6–58.2 mm in females, it is distinguished from five congeners, including *Boulenophrys caudoprocta* (66.2–81.3 mm in males; SVL 77.8 mm in female), *B. liboensis* (60.5–67.7 mm in males), *B. mirabilis* (SVL 55.8–61.4 mm in males; 68.5–74.8 mm in females), *B. omeimontis* (52.1–62.0 mm in males; 65.7–71.2 mm in females), and *B. shuichengensis* (102.0–118.3 mm in males), all of which are considerably larger.

The new species lacks vomerine teeth, distinguishing it from 25 congeners, *Boulenophrys daiyunensis*, *B. daweimontis*, *B. dongguanensis*, *B. elongata*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. hoanglienensis*, *B. insularis*, *B. jingdongensis*, *B. jinggangensis*, *B. jiulianensis*, *B. lichun*, *B. lushanensis*, *B. nankunensis*, *B. nanlingensis*, *B. nebulosa*, *B. palpebralespinosa*, *B. pepe*, *B puningensis*, *B. qianbeiensis*, *B. rubrimera*, *B. shimentaina*, *B. tongboensis*, and *B. yingdeensis* (vs. presence of vomerine teeth).

The new species can be easily distinguished from 15 other congeners by its relatively longer shanks and overlapping of its heels when the flexed hindlimbs are held at right angles to the body axis, differing from *Boulenophrys acuta*, *B. brachykolos*, *B. daoji*, *B. gaolanensis*, *B. hengshanensis*, *B. huitai*, *B. kuatunensis*, *B. obesa*, *B. ombrophila*, and *B. wugongensis* (vs. relatively shorter shanks with the heels not meeting). *B. binchuanensis*,

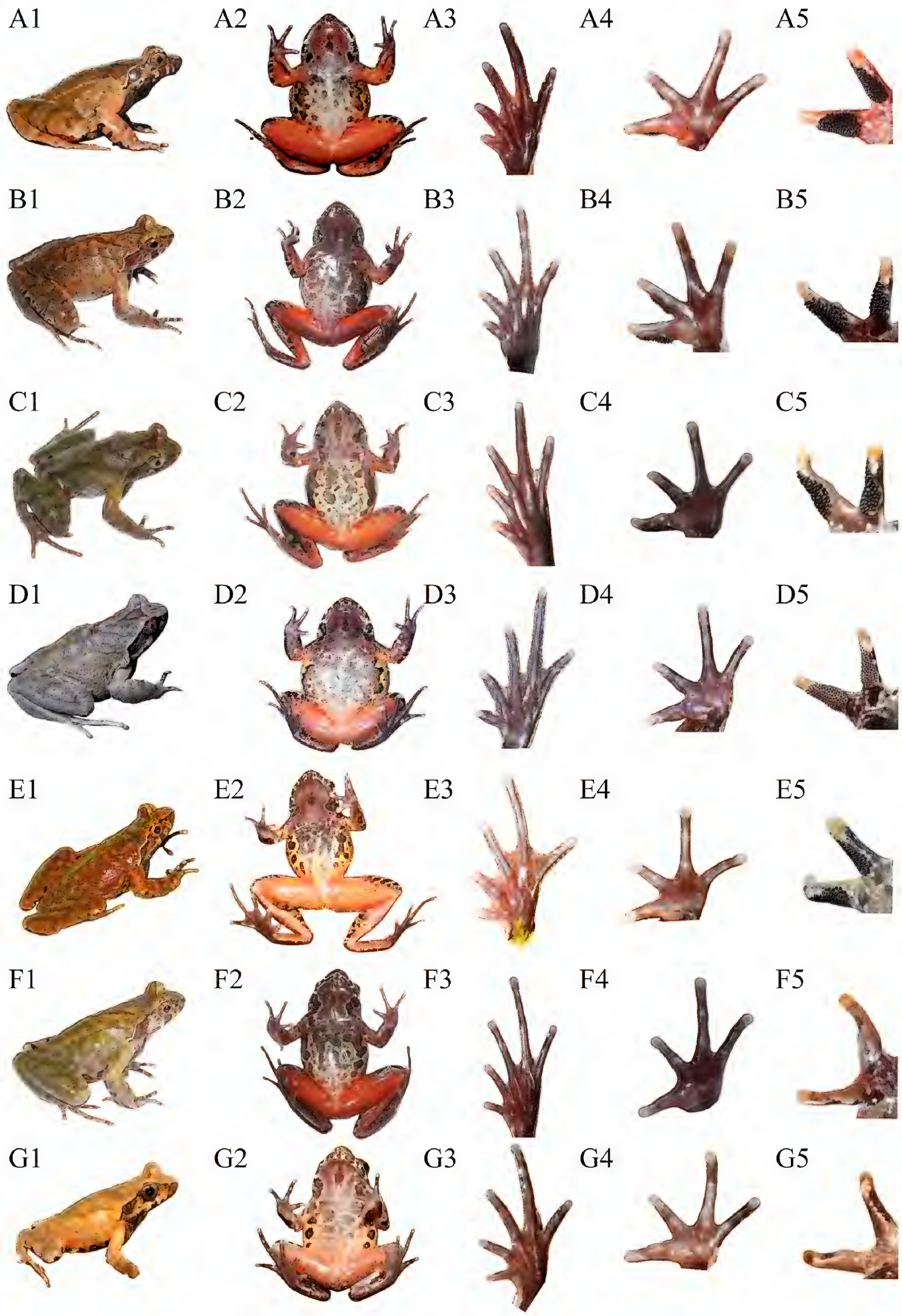

Figure 10. Morphological comparisons in adult males of *Boulenophrys youran* sp. nov. and its relative species. 1. lateral or dorsolateral body, 2. ventral body, 3. ventral foot, 4. ventral hand, 5. nuptial pads and spines on dorsal of inner two fingers (not to scale). **A.** *B. youran* sp. nov. (HUNL XB25061603, paratype from Nanshan National Park, Hunan, China); **B.** *B. dalaolingensis* (CIB YL20240518023, topotype from Dalaoling Nature Reserve, Hubei, China); **C.** *B. fanjingmontis* (unvouchered individual from type locality Mt. Fanjing, Tongren, Guizhou, China); **D.** *B. qianbeiensis* (CIB WB2020211, topotype from Tongzhi,, Guizhou, China); **E.** *B. spinata* (CIB LS20190801002, topotype from Mt. Leigong, Guizhou, China); **F.** *B. sangzhiensis* (CIB TPS20190625004, topotype from Mt. Tianping, Hunan, China); **G.** *B. yezhongensis* (CIB SC20240531005, holotype from Yezhong Nature Reserve, Guizhou, China). Photographer: **A** by Hui Li; **B, C, D, F** by Shengchao Shi; **E, G** by Shize Li.

B. lishuiensis, *B. tuberogranulata*, *B. xiangnanensis*, *B. xuefengmontis* (vs. just meeting).

The new species possesses wide lateral fringes and one-third webbing, differing from 28 other congeners *Boulenophrys angka*, *B. baishanzuensis*, *B .baolongensis*, *B. changyangensis*, *B. chishuiensis*, *B. daxuemontis*, *B. huangniushiensis*, *B. jiangi*, *B. wuliangshanensis* (vs absence of toes webbing); as well as from *B. anlongensis*, *B. boettgeri*, *B. cheni*, *B. caobangensis*, *B. congjiangensis*, *B. dupanglingensis*, *B. gutu*, *B. lini*, *B. leishanensis*, *B. lushuiensis*, *B. minor*, *B. mufumontana*, *B. sanmingensis*, *B. shunhuangensis*, *B. wushanensis*, *B. xianjuensis*, *B. yangmingensis*, *B. yaoshanensis* and *B. yunkaiensis* (vs. toes with rudimentary webbing).

Finally, the new species is characterized by tibiotarsal articulation exceeding the snout tip when the leg stretched forward, distinguishing it from *Boulenophrys binlingensis*, *B. dalaolingensis* and *B. liboensis* (vs. its reaches below the snout tip). Diagnostic characters distinguishing the new species from all recognized *Boulenophrys* congeners are presented in Suppl. material 1: table S2.

Distribution and habits. *Boulenophrys youran* sp. nov. is currently known from Chengbu Miao Autonomous County and Xinning County, Hunan Province, China (1 040–1 750 m a.s.l.). The new species inhabits flowing montane streams and nearby forest floors within subtropical evergreen broad-leaved forest. It was frequently encountered near streams (Fig. 11), in sympatry with *B. shunhuangensis*. Females with mature eggs and

Figure 11. Habitat of *Boulenophrys youran* sp. nov. in Nanshan National Park, Shaoyang City, Hunan Province, China. **A.** Microhabitats of the new species, mountain stream in Nanshan National Park; **B.** Male paratype HUNL XB24052601 found calling on rocks near stream *in situ*; **C.** Montane forests and stream at the type locality. Photographed by Leqiang Zhu.

calling males were found from April to June. Notably, nuptial pads of males collected in September without distinct nuptial spines, suggesting a period of reproductive inactivity during that time.

Discussion

The description of *Boulenophrys youran* sp. nov. further highlights the diversity of the genus in Hunan Province. The transitional zone between the Nanling Mountains and Xuefeng Mountains in southwestern Hunan, covered with subtropical montane forests, lies within one of the biodiversity-rich but underexplored regions identified by Xu et al. (2024). Our finding further shed light on the amphibian diversity of this topographically complex landscape, where cryptic species were expected to be found (Olave et al. 2017; Knight et al. 2021).

The PCA analysis based on shared measurements for the new species and its close related species could not separate it from all other congeners. This implies the convergence for their morphology, which has long been founded in this rapid radiation genus (Liu et al. 2018; Lyu et al. 2023). However, sexually dimorphic characters like nuptial spines on fingers of breeding males were not included in the morphometric analysis, which often provide more reliable diagnostic value than general body proportions in species delimitation.

Rapid radiation occurring over short evolutionary timescales can hinder complete lineage sorting, preventing alleles from reaching random fixation across species (Doronina et al. 2017; Black et al. 2024). Within the *Boulenophrys omeimontis* species group, limited genetic divergence in mitochondrial 16S rRNA, exemplified by remarkably low interspecific distances, for instance, 0.3% between *B. yezhongensis* and *B. dalaolingensis*, 0.9% between *B. yezhongensis* and *B. qianbeiensis*. Nevertheless, these taxa exhibit pronounced morphological differentiation, supporting their recognition as new species. It is likely attributable to the limited phylogenetic signal of this gene fragment, and the fast speciation rate for the genus (Liu et al. 2018; Chan et al. 2022).

Boulenophrys youran sp. nov. occurs sympatrically with *B. shunhuangensis* in Nanshan National Park, phylogenetic analyses place them in distinct clades, our investigation found that they occupy different microhabitats: the smaller sized *B. shunhuangensis* preferentially inhabits the headwaters of narrow montane streams, whereas *B. youran* sp. nov. currently is only found in Nanshan National Park, where their habitats were mostly included in the protected area. However, due to its narrow distribution, we recommend further investigation on its population size and ecology, to better estimate its conservation status.

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References

Black AN, Heenkenda EJ, Mathur S, Willoughby JR, Pierce BL, Turner SJ, Rizzuto D, DeWoody JA (2024) Rapid vertebrate speciation via isolation, bottlenecks, and drift. Proceedings of the National Academy of Sciences of the United States of America 121(22): 2320040121. https://doi.org/10.1073/pnas.2320040121

Boulenger GA (1899) On a collection of reptiles and batrachians made by Mr. J. D. La Touche in N.W. Fokien, China. Proceedings of the Zoological Society of London 1899: 159–172. https://doi.org/10.1111/j.1469-7998.1899.tb06855.x

Chan KO, Hertwig ST, Neokleous DN, Flury JM, Brown RM (2022) Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. BMC Ecology and Evolution 22(1): 37. https://doi.org/10.1186/s12862-022-01994-y

Chen JM, Zhou WW, Poyarkov Jr NA, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZY, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen SN, Duong TV, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys sensu lato* (Anura: Megophryidae). Molecular Phylogenetics and Evolution 106: 28–43. https://doi.org/10.1016/j.ympev.2016.09.004

Doronina L, Churakov G, Kuritzin A, Shi J, Baertsch R, Clawson H, Schmitz J (2017) Speciation network in Laurasiatheria: Retrophylogenomic signals. Genome Research 27(6): 997–1003. https://doi.org/10.1101/gr.210948.116

Dubois A, Ohler A, Pyron RA (2021) New concepts and methods for phylogenetic taxonomy and nomenclature in zoology, exemplified by a new ranked cladonomy of recent amphibians (Lissamphibia). Megataxa 5(1): 1–738. https://doi.org/10.11646/megataxa.5.1.1

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Fei L, Ye CY (2016) Amphibians of China, Vol. 1. Science Press, Beijing, China.

Fei L, Ye CY, Huang YZ (1983) Two new subspecies of *Megophrys omeimontis* Liu from China (Amphibia, Pelobatidae). Acta Herpetologica Sinica. New Series, Chengdu 2(2): 49–52. [In Chinese]

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia (Vol. 2). Anura. Science Press, Beijing.

Frost DR (2025) Amphibian Species of the World: an Online Reference. Version 6.2 (Date of access). Electronic Database. https://amphibiansoftheworld.amnh.org/index.php [Accessed 19 November, 2025]

Gao Z, Qian T, Jiang J, Hou D, Deng X, Yang D (2022) Species diversity and distribution of amphibians and reptiles in Hunan Province, China. Biodiversity Science 30(2): 21290. https://doi.org/10.17520/biods.2021290

Hu SX, Zhao EM, Liu CZ (1973) A survey of amphibians and reptiles in Kweichow province, including a herpetofauna analysis. Acta Zoologica Sinica 19(2): 149–171.

Jiang JP, Ye CY, Fei L (2008) A New Horn Toad *Megophrys sangzhiensis* from Hunan, China (Amphibia, Anura). Zoological Research 29(2): 219–222. https://doi.org/10.3724/SP.J.1141.2008.00219

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Knight DR, Imwattana K, Kullin B, Guerrero-Araya E, Paredes-Sabja D, Didelot X, Dingle KE, Eyre DW, Rodríguez C, Riley TV (2021) Major genetic discontinuity and novel toxigenic species in Clostridioides difficile taxonomy. eLife 10: e64325. https://doi.org/10.7554/eLife.64325

Kuang D, Wei Y, Luo Y, Pei K, Cao Y, Zhang M, Huang T, Pu L, Shi S (2025) A New Species of *Boulenophrys* (Megophryidae) from Mt. Hengshan, Hunan Province, China, with Re – Description on B. hengshanensis. Animals 15(18): 2745. https://doi.org/10.3390/ani15182745

Li YL, Jin MJ, Zhao J, Liu ZY, Wang YY, Pang H (2014) Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Nature Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. Zootaxa 3795(4): 449–471. https://doi.org/10.11646/zootaxa.3795.4.5

Li SZ, Xu N, Liu J, Jiang JP, Wei G, Wang B (2018) A New Species of the Asian Toad Genus *Megophrys sensu lato* (Amphibia: Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 9(4): 224–239. https://doi.org/10.16373/j.cnki.ahr.180072

Li SZ, Lu NN, Liu J, Wang B (2020) Description of a new *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from Guizhou Province, China. ZooKeys 986: 101–126. https://doi.org/10.3897/zookeys.986.57119

Li SZ, Shi SC, Liu J, Zhao JJ, Gao S, Wang B (2025) A new species of the *Boulenophrys* (Anura, Megophryidae) from Hubei, China. Zoosystematics and Evolution 101(3): 1213–1226. https://doi.org/10.3897/zse.101.155859

Lin SS, Chen HH, Li YH, Peng ZN, Zeng ZC, Wang J (2024) A new *Boulenophrys* species (Anura, Megophryidae) from the coastal hills of eastern Fujian Province, China. ZooKeys 1216: 1–15. https://doi.org/10.3897/zookeys.1216.130017

Liu ZY, Zhu TQ, Zeng ZC, Lyu ZT, Wang J, Messenger K, Greenberg AJ, Gou ZX, Yang ZH, Shi SH, Wang YY (2018) Prevalence of cryptic species in morphologically uniform taxa–Fast speciation and evolutionary radiation in Asian frogs. Molecular Phylogenetics and Evolution 127: 723–731. https://doi.org/10.1016/j.ympev.2018.06.020

Liu J, Li SZ, Wei G, Xu N, Cheng YL, Wang B, Wu J (2020) A New Species of the Asian Toad Genus *Megophrys sensu lato* (Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 11(1): 1–18. https://doi.org/10.16373/j.cnki.ahr.190041

Liu J, Feng CB, Shen T, Li SZ, Cheng YL, Wei G, Wang B, Su HJ (2025a) A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Guizhou, China. Herpetozoa 38: 117–136. https://doi.org/10.3897/herpetozoa.38.e155430

Liu J, Li SZ, Cheng YL, Wei G, Wang B, Cheng G (2025b) A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Southwest China. Biodiversity Data Journal 13: e153987. https://doi.org/10.3897/BDJ.13.e153987

Lleonart J, Salat J, Torres GJ (2000) Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205(1): 85–93. https://doi.org/10.1006/jtbi.2000.2043

Luo T, Wang Y, Wang S, Lu X, Wang W, Deng H, Zhou J (2021) A species of the genus *Panophrys* (Anura, Megophryidae) from southeastern Guizhou Province, China. ZooKeys 1047: 27–60. https://doi.org/10.3897/zookeys.1047.61097

Lyu ZT, Li YQ, Zeng ZC, Zhao J, Liu ZY, Guo GX, Wang YY (2020) Four new species of Asian horned toads (Anura, Megophryidae, Megophrys) from southern China. ZooKeys 942: 105–140. https://doi.org/10.3897/zookeys.942.47983

Lyu ZT, Zeng ZC, Wang J, Liu ZY, Huang YQ, Li WZ, Wang YY (2021) Four new species of *Panophrys* (Anura, Megophryidae) from eastern China, with discussion on the recognition of Panophrys as a distinct genus. Zootaxa 4927(1): 009–040. https://doi.org/10.11646/zootaxa.4927.1.2

Lyu ZT, Qi S, Wang J, Zhang SY, Zhao J, Zeng ZC, Wan H, Yang JH, Mo YM, Wang YY (2023) Generic classification of Asian horned toads (Anura: Megophryidae: Megophryinae) and monograph of Chinese species. Zoological Research 44(2): 380–450. https://doi.org/10.24272/j.issn.2095-8137.2022.372

Mahony S, Sengupta S, Kamei RG, Biju SD (2011) A new low altitude species of *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae), from Assam, Northeast India. Zootaxa 3059(1): 36–46. https://doi.org/10.11646/zootaxa.3059.1.2

Mahony S, Nicole MF, Biju SD, Teeling EC (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): Integrative approaches to timetree dating in the absence of a fossil record. Molecular Biology and Evolution 34(3): 744–771. https://doi.org/10.1093/molbev/msw267

Messenger KR, Dahn HA, Liang Y, Xie P, Wang Y, Lu C (2019) A new species of the genus *Megophrys* Gunther, 1864 (Amphibia: Anura: Megophryidae) from Mount Wuyi, China. Zootaxa 4554(2): 561–583. https://doi.org/10.11646/zootaxa.4554.2.9

Meyer CP, Geller JB, Paulay G (2005) Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. Evolution; International Journal of Organic Evolution 59(1): 113–125. https://doi.org/10.1111/j.0014-3820.2005.tb00899.x

Minh BQ, Nguyen MA, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

Mo XY, Shen YH, Li HH, Wu XS (2010) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from the northwestern Hunan Province, China. Current Zoology 56(4): 432–436. https://doi.org/10.1093/czoolo/56.4.432

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ – TREE: A fast and effective stochastic algorithm for estimating maximum – likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Nguyen TQ, Pham CT, Nguye TT, Luong AM, Ziegler T (2020) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from Vietnam. Zootaxa 4722(5): 401–422. https://doi.org/10.11646/zootaxa.4722.5.1

Olave M, Avila LJ, Sites Jr JW, Morando M (2017) Hidden diversity within the lizard genus *Liolaemus*: Genetic vs morphological divergence in the *L. rothi* complex (Squamata:Liolaeminae). Molecular Phylogenetics and Evolution 107: 56–63. https://doi.org/10.1016/j.ympev.2016.09.009

Qi S, Lyu ZT, Wang J, Mo YM, Zeng ZC, Zeng YJ, Dai KY, Li YQ, Grismer LL, Wang YY (2021) Three new species of the genus *Boulenophrys* (Anura, Megophryidae) from southern China. Zootaxa 5072(5): 401–438. https://doi.org/10.11646/zootaxa.5072.5.1

Qian TY, Hu K, Mo XY, Gao ZW, Zhang N, Yang DD (2023) A new species of *Boulenophrys* from central Hunan Province, China (Anura: Megophryidae). Vertebrate Zoology 73: 915–930. https://doi.org/10.3897/vz.73.e100889

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Shen YH, Yang DD, Mo XY, Li HH, Chen D (2014) The Fauna of Hunan: Amphibia. Hunan Science and Technology Press, Changsha.

Shen DM, Zhou HY, Wang CY, Xu J, Xu S, Zhong YF, Wang ZY, Cai H, Wu YH, Wei MC, Li YK (2025a) A New Species of *Boulenophrys* (Anura, Megophryidae) from Northern Jiangxi, China. Zoosystematics and Evolution 101(4): 2251–2266. https://doi.org/10.3897/zse.101.166556

Shen DM, Zhou HY, Messenger KR, Amin H, Wang ZY, Xu J, Xu S, Li YK (2025b) A New Species of *Boulenophrys* (Anura, Megophryidae) from Northern Jiangxi, China. Animals 15(21): 3197. https://doi.org/10.3390/ani15213197

Shi SC, Li DH, Zhu WB, Wang J, Jiang JP, Ye CY, Fei L, Wang B (2021) Description of a new toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Amphibia: Anura: Megophryidae) from western Yunnan Province, China. Zootaxa 4942(3): 351–381. https://doi.org/10.11646/zootaxa.4942.3.3

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Song HM, Wang HT, Qi S, Wang N, Wang YY (2024) A new species of the genus *Boulenophrys* (Anura: Megophryidae: Megophryinae) from Zhuhai, Guangdong, China. Asian Herpetological Research 15(4): 251–264. https://doi.org/10.3724/ahr.2095-0357.2024.0026

Su HJ, Shi SC, Wu YQ, Li GG, Yao XG, Wang B, Li SZ (2020) Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. ZooKeys 974: 131–159. https://doi.org/10.3897/zookeys.974.56070

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Nguyen TT, Luong HV, Rowley JJL (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa 4344(3): 465–492. https://doi.org/10.11646/zootaxa.4344.3.3

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Luong AM, Le DT, Nguyen TT, Nguyen TQ, Portway C, Luong HV, Rowley JJL (2018a) Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa 4508(3): 301–333. https://doi.org/10.11646/zootaxa.4508.3.1

Tapley B, Cutajar T, Nguyen LT, Nguyen CT, Harding L, Portway C, Van Luong H, Rowley JJL (2018b) A new locality and elevation extension for *Megophrys rubrimera* (Tapley et al., 2017) in Bat Xat Nature Reserve, Lao Cai Province, northern Vietnam. Herpetology Notes 11: 865–868.

Tapley B, Cutajar T, Nguyen LT, Portway C,Mahony S, Nguyen CT, Harding L, Luong HV, Rowley JJL (2020) A new potentially Endangered species of *Megophrys* (Amphibia: Megophryidae) from Mount Ky Quan San, north-west Vietnam. Journal of Natural History 54(39–40): 2543–2575. https://doi.org/10.1080/00222933.2020.1856952

Tian YZ, Gu XM, Sun AQ (2000) A new species of *Xenophrys* in China (Amphibia: Pelobatidae). Acta Zootaxonomica Sinica 25: 462–466. [In Chinese]

Wang YY, Zhang TD, Zhao J, Sung YH, Yang JH, Pang H, Zhang Z (2012) Description of a new species of the genus *Xenophrys* Gunther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa 3546(1): 53–67. https://doi.org/10.11646/zootaxa.3546.1.4

Wang YY, Zhao J, Yang JH, Zhou ZM, Chen GL, Liu Y (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Megophrys* (Amphibia: Anura Megophryidae) species in Southeast China. PLoS ONE 9(4): e93075 [1–15]. https://doi.org/10.1371/journal.pone.0093075

Wang J, Liu ZY, Lyu ZT, Zeng ZC, Wang YY (2017a) A new species of the genus *Xenophrys* (Amphibia: Anura: Megophryidae) from an offshore island in Guangdong Province, southeastern China. Zootaxa 4324(3): 541–556. https://doi.org/10.11646/zootaxa.4324.3.8

Wang YE, Liu BQ, Jiang K, Jin W, Xu JN, Wu CH (2017b) A new species of the Horn Toad of the genus *Xenophrys* from Zhejiang, China (Amphibia: Megophryidae). Chinese Journal of Zoology 52: 19–29. [in Chinese with English abstract]

Wang J, Lyu ZT, Liu ZY, Liao CK, Zeng ZC, Zhao J, Li YL, Wang YY (2019a) Description of six new species of the subgenus *Panophrys* within the genus Megophrys (Anura, Megophryidae) from southeastern China based on molecular and morphological data. ZooKeys 851: 113–164. https://doi.org/10.3897/zookeys.851.29107

Wang L, Deng XJ, Liu Y, Wu QQ, Liu Z (2019b) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Hunan, China. Zootaxa 4695(4): 301–330. https://doi.org/10.11646/zootaxa.4695.4.1

Wang B, Wu YQ, Peng JW, Shi SC, Lu NN, Wu J (2020) A new *Megophrys* Kuhl & Van Hasselt (Amphibia, Megophryidae) from southeastern China. ZooKeys 904: 35–62. https://doi.org/10.3897/zookeys.904.47354

Wang J, Zeng ZC, Lyu ZT, Qi S, Liu ZY, Chen HH, Lu YH, Xiao HW, Lin CR, Chen K, Wang YY (2022) Description of three new *Boulenophrys* species from eastern Guangdong, China, emphasizing the urgency of ecological conservation in this region (Anura, Megophryidae). Zootaxa 5099(1): 91–119. https://doi.org/10.11646/zootaxa.5099.1.4

Wang J, Lin SS, Gan JS, Chen HH, Yu LM, Pan Z, Xiao JJ, Zeng ZC (2024) A new species of the genus *Boulenophrys* from South China (Anura, Megophryidae). Zootaxa 5514(5): 451–468. https://doi.org/10.11646/zootaxa.5514.5.3

Wang HT, Wu XG, Song HM, Zhuo XH, Wang YY (2025a) A new species of the genus *Boulenophrys* (Anura: Megophryidae: Megophryinae) from Jiulianshan National Nature Reserve, southern Jiangxi, China. Zootaxa 5691(3): 376–398. https://doi.org/10.11646/zootaxa.5691.3.1

Wang R, Chen L, Chen J, Huang H, Peng G, Peng H, Gao X, Chen B, Liu N (2025b) A new horned toad of *Boulenophrys* (Anura, Megophryidae) was discovered in Hubei Province, China. ZooKeys 1253: 305–320. https://doi.org/10.3897/zookeys.1253.154757

Wu YH, Suwannapoom C, Poyarkov Jr NA, Pawangkhanant P, Xu K, Jin JQ, Murphy RW, Che J (2019) A new species of the genus *Xenophrys* Anura Megophryidae from northern Thailand. Zoological Research 40(6): 564–574. https://doi.org/10.24272/j.issn.2095-8137.2019.032

Wu Y, Li S, Liu W, Wang B, Wu J (2020) Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Amphibia, Megophryidae) from Zhejiang Province, China. ZooKeys 1005: 73–102. https://doi.org/10.3897/zookeys.1005.58629

Xiang H, Zhou Q, Li W, Shu J, Gu Z, Jiang W (2024) Insights into phylogenetic positions and distribution patterns: Complete mitogenomes of two sympatric Asian horned toads in *Boulenophrys* (Anura: Megophryidae). Ecology and Evolution 14(7): e11687. https://doi.org/10.1002/ece3.11687

Xiao B, Xi JY, Shi SC, Li H, Zhu LQ, Maimaiti A, Xu YL, Liao SH, Wang B, Mo XY (2025) A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Southern Hunan Province, Central China. Animals 15(3): 440. https://doi.org/10.3390/ani15030440

Xu N, Li SZ, Liu J, Wei G, Wang B (2020) A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. ZooKeys 943: 119–144. https://doi.org/10.3897/zookeys.943.50343

Xu W, Wu YH, Zhou WW, Chen HM, Zhang BL, Chen JM, Xu W, Rao DQ, Zhao H, Yan F, Yuan Z, Jiang K, Jin JQ, Hou M, Zou D, Wang LJ, Zheng Y, Li JT, Jiang J, Zeng XM, Chen Y, Liao ZY, Li C, Li XY, Gao W, Wang K, Zhang DR, Lu C, Yin T, Ding Z, Zhao GG, Chai J, Zhao WG, Zhang YP, Wiens JJ, Che J (2024) Hidden hotspots of amphibian biodiversity in China. Proceedings of the National Academy of Sciences of the United States of America 121(20): e2320674121. https://doi.org/10.1073/pnas.2320674121

Ye CY, Fei L (1995) Taxonomic studies on the small type *Megophrys* in China including descriptions of the new species (subspecies) (Pelobatidae: genus *Megophrys*). Herpetologica Sinica 4–5: 72–81. [In Chinese]

Ye CY, Fei L, Xie F (2007) A new species of Megophryidae–*Megophyrys baolongensis* from China (Amphibia, Anura). Acta Herpetologica Sinica 11: 38–41. [In Chinese].

Zeng ZC, Wang J, Chen HH, Xiao WW, Zhan BB, Li YH, Lin SS (2024) A New Species of the Genus *Boulenophrys* (Anura, Megophryidae) from Eastern Guangdong, China. Asian Herpetological Research 15(1): 12–21. https://doi.org/10.3724/ahr.2095-0357.2023.0038

Zhang L, Liang L, Ran H, Sheng ZX (2012) *Megophrys binlingensis fanjingmontis*: A New Subspecies of Megophryidae from Guizhou, China. Chinese Journal of Zoology 47(4): 135–138. https://doi.org/10.13859/j.cjz.2012.04.020

Zhang YN, Li G, Xiao N, Li JQ, Pan T, Wang H, Zhang BW, Zhou J (2017) A new species of the genus *Xenophrys* (Amphibia: Anura: Megophryidae) from Libo County, Guizhou, China. Asian Herpetological Research 8(2): 75–85. https://doi.org/10.16373/j.cnki.ahr.160041

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Appendix 1

Specimens examined for comparisons.

Boulenophrys dalaolingensis, CIB YL20240518023, male topotype from Dalaoling Nature Reserve, Hubei, China.

Boulenophrys fanjingmontis, unvouchered male from type locality Mt. Fanjing, Tongren, Guizhou, China.

Boulenophrys qianbeiensis, CIB WB2020211, male topotype from Tongzi, Guizhou, China.

Boulenophrys spinata, CIB LS20190801002, male topotype from Mt. Leigong, Guizhou, China.

Boulenophrys sangzhiensis, CIB TPS20190625004, male topotype from Mt. Tianping, Hunan, China).

Boulenophrys yezhongensis, CIB SC20240531005, male holotype from Yezhong Nature Reserve, Guizhou, China.

Supplementary material 1

Supplementary information

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